ORIGINAL ARTICLE

CONTENTS

The investigation effect of storage place on bacterial contamination toothbrushes

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ABSTRACT

Aim: Investigate and compare microbial contamination of toothbrushes, which kept in bathrooms with/without attached toilet.

Materials and Methods: Thirty adults (15 in each group), aged 18-25 years, requested to follow their normal oral hygiene practice for 3 months period. Research was carried out between January to June 2020. The 1st group kept brush in bathroom with attached toilet, on the other hand the 15 brushes were kept in bathroom without attached toilet after usage, and using different media (blood agar, MacConkay agar, Mantiol agar, E.M.B agar), incubated in 37c for 24 hours, some plats (blood agar) incubated in Candle jar 5%CO2 about 48-72 hours.

Results: 26(86.6%) of toothbrushes collected from samples were found to be contaminated with usual oral bacteria and 40(61.5%) isolated from toilets G1 and 25(38.4%) in bathrooms G2. A 34(51%) were found to be growth positive and 31(48%) were negative bacteria, were fairly close in both groups. Toothbrush was kept in bathrooms without attached toilets. Common Gram-positive bacterial isolates were *Streptococcus spp*. 36%, and *Staphylococcus spp*. 8%. Whereas *Pseudomonas spp.* 28%, while *Klebsiella spp*., *Escherichia coli* recorded 20%, 8% respectively. Toothbrush was kept in bathrooms with attached toilets was *Streptococcus spp. 27%, and Staphylococcus spp.,* showed nearly percentage 22%, while *Klebsiella spp*., *E. coli* 17.5 %, 10% respectively.

Conclusions: Toothbrushes should not be kept in bathrooms with/without attached toilets as it is prone for contamination, and should be kept in any type disinfectants such as 0.2% chorhexidine gluconate or 3% hydrogen peroxide for prevention of future infection.

KEY WORDS: storage, bacterial infection, toothbrushes, toilets

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INTRODUCTION

The mouth serves as a clear window to the rest of the human body, which gives a general picture and indication of health disorders. There are several surfaces of the oral cavity: teeth, saliva, mucous membranes, gingival groove, and tongue [1]. Each of them is a good environment to host different types of microorganisms. Here it has become necessary to take care of it, maintain its integrity as a tissue, and easily get rid of pathogens such as viruses, bacteria, fungi, etc. [2]. The oral cavity is the simplest and fastest place for microorganisms to move and enter through the process of eating, drinking, breathing, in addition to brushing the teeth, which may lead to many bacterial infection and other diseases, such as pharynx, larynx, digestive system, etc. [3]. Currently, oral care and the oral cavity require only a few minutes a day, as an essential and necessary part of an individual's systemic health public welfare [4-5]. That protects people from diseases such as tooth caries and periodontal diseases, and others due to microorganisms, which gives a clear tangible change to human health [6]. More than 700 bacterial species have been found in the mouth. Here comes the role of the toothbrush, which is considered one of the essential equipment for effective and easy oral care [7]. If the toothbrush is used correctly, then it will mechanically remove microorganisms, debris and food residue from the oral cavity. Hence that was main purpose of using a toothbrush [3]. While that vital daily tool can be contaminated and transmit microorganisms and thus become a potential source of infection and individual contamination [8-9]. Not only organisms they are related with the oral cavity may also seem in the toothbrush containing *Staphylococcus aureus, Streptococcus mutans, Klebsiella, Lactobacillus, Pseudomonas*, Candida species. Toothbrushes can become polluted not only from the oral cavity but also hand, aerosol contamination, environment, moreover storage containers and the bacteria simply which attach to the toothbrush develops accumulated and survive on toothbrushes will assistance in spreading the diseases [10]. It is worth noting that the toothbrush can become

contaminated in the bathroom itself or outside, and in the case of erratic aerosols released from the toilet [11-13]. The toothbrush is usually stored in the bathroom, and perhaps next to the toilet as well as the sink. Here, it is likely to be exposed to various types and forms of harmful and harmless bacteria, could also the rest of the microorganisms [4, 11]. That known a humid environment is more suitable for growth of different microorganisms to open air [10, 14]. It is not known that if a toothbrush is placed in the bathroom will be contaminated with bacteria than placed in the toilet. Regrettably, we have a clear lack of experience as well as a deficiency of awareness among many people regarding the correct and proper care and maintenance of a toothbrush.

AIM

Investigate and compare microbial contamination of toothbrushes, which kept in bathrooms with/without attached toilet.

MATERIAL AND METHODS

Thirty adults (15/group), between the ages of 18 and 25, were given a new toothbrush of the same brand and type as well as identical tubes of fluoridated toothpaste. They were asked to follow normal oral hygiene practices for 3 months and not to take any antimicrobial medication during this study period. This study was conducted in the Department of Microbiology, College of Dentistry, University of Kufa, in the period from January to June 2020, where thirty brushes were collected from adults. The brushes in the first group (G1) 15 adults were kept in the bathroom attached to the toilet surface. On the other hand, the 15 (G2) brushes were kept in the bathroom without the attached toilet after use. Then each toothbrush and its 30 bristles were collected in a sterile plastic container. While maintaining sterile measures and adhering to strict protocol, toothbrush bristles were cut using sterile scissors and dropped into the brain heart infusion broth, which supports bacterial growth and incubated for 24 hours at 37°C. All media were prepared according to the manufacturer's instructions using different media (blood agar, MacConay agar, menthol agar, E.M.B agar) and incubated aerobically at 37°C for 24 h. Some plates (blood agar) were incubated in a candle jar (5% CO2) for approximately 48-72 hours. In addition, isolates were identified by Gram stain, and biochemical testing was used to diagnose bacteria according to the standard bacteriological protocol [15-16].

RESULTS

This prospective study was conducted in the Basic Sciences Laboratory, College of Dentistry, University of Kufa, for a period of 6 months, to detect and investigate the bacterial founding in the toothbrushes of 30 people in two groups, half of them were in the bathrooms and the other in the toilet. The finding of the current study revealed bacterial growth in 26(86.6%) sample, while

Table 1. Isolation of bacteria after 3 months, toothbrushes kept in the bathroom without attached toilet (G2)

No. of toothbrushes	*Streptococcus ssp.*	*Staphylococcus ssp.*	*E. coli*	*Klebsiella spp.*	*Pseudomonas spp.*
1	-	-	-	-	-
2	+	-	+	-	+
3	+	-	-	+	+
4	+	-	-	+	+
5	-	-	-	-	+
6	-	-	-	+	+
7	+	+	-	-	-
8	+	-	-	-	+
9	-	-	+	-	-
10	+	+	-	-	-
11	+	-	-	-	+
12	-	-	-	-	-
13	+	-	-	+	-
14	+	-	-	+	-
15	-	-	-	-	-
total	9(36%)	2(8%)	2(8%)	5(20%)	7(28%)

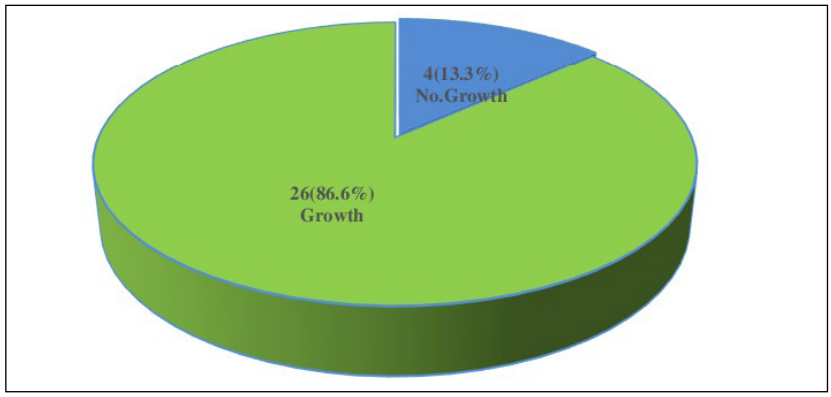

Fig. 1. Pattern of growth and non-growth (n=30)

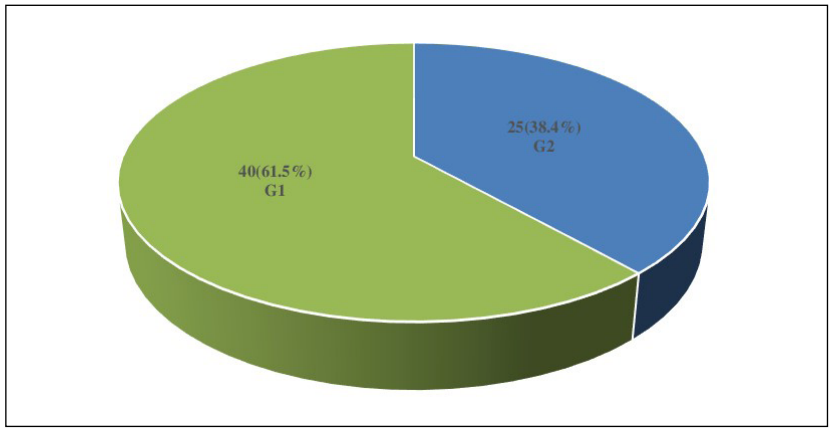

Fig. 2. Bacterial growth Pattern of bathroom with and without toilet (G1 and G2) (n=65)

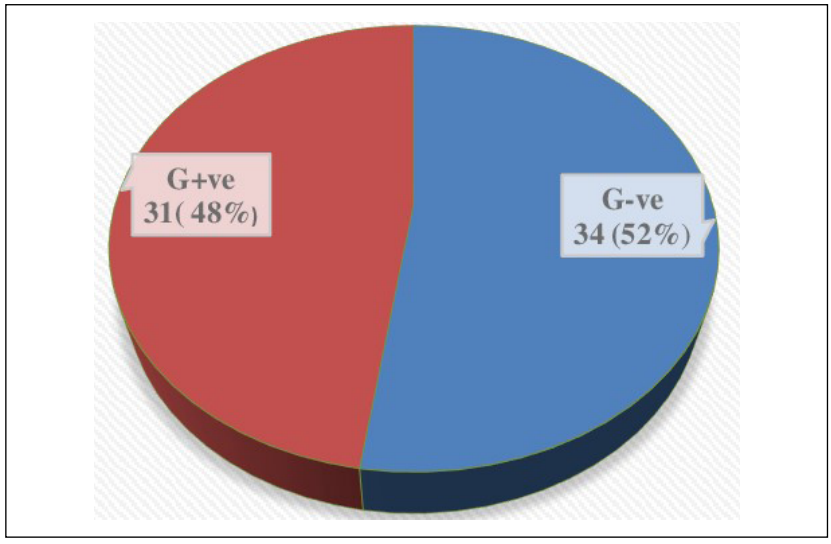

Fig. 3. Percentage of Gram negative and positive bacteria in both groups (G1and G2) N=65

4(13.3%) samples only did not grow out of the total study samples, despite the strict sterilization conditions and the identical sample collection method as in the Fig. 1. Also, the total percentage of bacteria samples isolated from toilets 40(61.5%) (G1) is higher in compared to that in bathrooms 25(38.4%) (G2), Fig. 2. In addition, the results with the numbers of negative and positive bacteria 31, 48% and 34, 51% were fairly close in both groups (G1 and G2) Fig. 3.

The table below table 1 demonstrates that the main isolates of bacteria when keeping toothbrushes in bathrooms without attached toilets (G2), *Streptococcus* and *Pseudomonas* were dominate. Both of these bacteria have a high percentage among other isolates, *Streptococcus spp*. 9(36%) as one of the most important Gram-positive bacteria, followed by *Pseudomonas* spp. 7(28%), while Klebsiella spp. 5(20%) however, *Staphylococcus* and *E. coli* species recorded 20%, 8%, and 8%, respectively.

On the other hand, the table 2 showed that the high percentage of bacteria when the toothbrush was kept in bathrooms with attached toilets (G1) was *Strepto-*

Table 2. Isolation of bacteria after 3 months, toothbrushes kept in the bathroom with attached toilet (G1)

No. of toothbrushes	*Streptococcus ssp.*	*Staphylococcus ssp.*	*E. coli*	*Klebsiella spp.*	*Pseudomonas spp.*
1	+	+	-	+	-
2	+	-	-	-	+
3	-	+	-	+	+
4	+	-	-	-	+
5	+	+	-	-	+
6	-	+	+	-	-
7	+	-	-	-	+
8	+	-	+	+	-
9	-	+	-	+	-
10	-	-	-	-	-
11	+	+	-	+	+
12	+	+	+	+	+
13	+	+	+	+	+
14	+	-	-	-	+
15	+	+	-	-	-
total	11(27.5%)	9(22.5%)	4(10%)	7(17.5%)	9(22.5%)

Table 3. Comparison between bacterial isolation after 3 months from toothbrushes kept in the bathroom with attached toilet (G1) and toothbrushes kept in the bathroom without attached toilet (G2) (No.)

Groups	***Streptococcus ssp.***	***Staphylococcus ssp.***	***E. coli***	***Klebsiella spp.***	***Pseudomonas spp.***
toothbrush kept in the bathroom with attached toilet (G1)	11	9	4	7	9
toothbrushes kept in the bathroom without attached toilet (G2)	9	2	2	5	7
Total (65)	20	11	6	12	16
P - value	Chi-square 2.58 df =4 p-value: 0.630				

coccus spp. 27%, *Staphylococcus spp*., *Pseudomonas spp*. Showed nearly percentage 22%, while *Klebsiella spp*., E. coli 17.5 %, 10% respectively.

However, the results shown in the table 3 below support that a high frequency of *Streptococcus spp.* 20 bacterial isolated in the bathroom with (G1) and without toilet (G2), 11 isolates to *Staphylococcus spp.* while *E. coli* showed low number 6 bacterial isolated in compared to other negative bacteria such as *Pseudomonas spp.* 16 isolate and *Klebsiella spp.*12 isolate. Also in the same table, there is no wide significant differences (P-value<0.05) between the bacterial growth in toothbrushes attached with toilet or without toilet.

DISCUSSION

Toothbrush contamination is an obvious and inevitable consequence of use improper storage leads to various and many systemic and oral infections in humans. In the following study, we have not studied a specific bacterium, were restricted ourselves to classifying them based on the contamination type. A total of 30 manual toothbrushes used over a 6-month period were obtained. They were divided into two groups, each group included 15 samples. In this investigation, among 30 toothbrush samples, 26 (86.6%), the high percentage were culture positive, gives importance to toothbrush storage, due to its effect on the transmission of bacteria [14], while 4 of them 13.3% were culture negative fig. 1. The culture may be negative due to the lack of appropriate conditions for bacteria to grow on the toothbrush or not using the toothbrush for an appropriate period or times, which is consistent with [3]. The results displayed that the place where the toothbrush is kept plays a fundamental role in its contamination and methods of transmission, and it is somewhat consistent with the results of [8]. Moreover, figure

(2) indicates that the high percentage (40, 61.5%) of toothbrush contamination came from its presence in bathrooms shared with toilets. The results were explained that it is inappropriate keeping a toothbrush in both bathrooms and toilets. Bacteria can partially transfer and stick to the toothbrush bristles to be a source of survival and infection; the result may agree with [17-18] they pointed out that the humid environment of the bathroom and toilets together encourages the bacterial growth. Among the results related to Figure 3, there was no high significant difference between Gram negative and positive bacteria that give us a perfect picture of the bathroom and toilet environment which encourages the bacterial growth of Gram negative and positive, may that not correspond with the findings of a previous study [19], which showed that although the toothbrush is not a suitable medium for bacterial growth, it is capable of sustaining bacterial life [20]. The current study showed the presence of negative and positive bacteria on the toothbrush in both groups (Tables (1-3), which contradicts the study conducted by [10], who conducted his study to verify the bacterial presence of the toothbrush and isolate three Gram positive species only, perhaps due to the small number of samples from five student volunteers were carried out in his study. Although, the numbers of positive and negative bacteria isolated from the toothbrush are somewhat similar in both groups table (3), *Streptococcus spp*., are relatively higher in numbers compared to the others. Here the results differ from those obtained by [18] was detected the superiority of *Staphylococci* and *Pseudomonas*, maybe because of the ability of these bacteria to form biofilms and adhere to toothbrush materials. These bacteria are part of the normal oral microflora, and are most commonly found in toothbrushes, which is somewhat consistent with [21]. The numbers of Streptococci spp. represent half of the bacterial isolates, which may give a similar picture to what was obtained [1], which considered them to be important factors causing the disease. On the other hand, *Staphylococcus spp.* that was not expected 11 isolates 16.9% were recorded compared to Streptococci spp. These deserve more attention because they are able to cause numerous body diseases through oral infection [4]. Despite its presence as a symbiont in the skin or transmitted through the hands that does not match the study conducted by [3], which obtained approximately half the number of isolates from these bacteria. The study documented that the toilet or bathroom also harbors another community of Gram-negative bacteria that can be partially transmitted to the toothbrush if kept in shared spaces. The findings showed the contamination of toothbrush with *Pseudomonas spp., Klebsiella spp*., and *E. coli*, that bacteria may cause various infections in the human body, such as upper respiratory tract infection, pneumonia, pyogenic infections, urinary tract infections, diarrhea, and septicemia. Here, the finding is consistent with the study conducted by [3,17]. A dominant group of bacteria has been identified was *Pseudomonas spp*. However, in this study, only 16(24.6%) of the total isolates identified were *Pseudomonas spp*., these bacteria are ubiquitous found everywhere in nature, including bathrooms and toilets, the result may be similar to what was mentioned [22] in his study, given that these bacteria are abundant in water sources used for cleaning, and here may be present in the toothbrush. The current study observed the isolation of members of the *Enterobacteriaceae* family, *Klebsiella* 12(18.4%) and *E. coli* 6(9.2%), despite of their small numbers, their contamination of the toothbrush is inevitable, as agreed with study [4], they grow well in hot, humid areas, such as those available in bathrooms and toilets, which shows the importance of preserving the toothbrush and placing it in the appropriate places to protect it from bacterial contamination, not just replacing the toothbrush every three or four months, that recommended by the American Dental Association [23-25]. The results of this study give a picture of the places where people in this study keep their toothbrushes on a daily and frequent basis, and the amount of bacterial contamination occurring there, which shows that bathrooms associated with environmental toilets are suitable for contaminating toothbrushes. They also speculate that the problem of bacterial contamination is great if toothbrushes are kept at a distance of less than one meter from the toilet, and that the reason is the aerosols dispersed in the place through the tank drain, and this has been confirmed by a number of studies [12, 26-29]. The results of this study concluded, the synergistic interactions between bathrooms associated with toilets are suitable for toothbrush contamination. It can be considered a vital part of its effectiveness for the spread and colonization of bacteria.

CONCLUSIONS

Toothbrushes should not be kept in the bathrooms with and without attached toilets as it is prone for contamination, and toothbrushes should be kept in any type disinfectants such as 0.2% chorhexidine gluconate or 3% hydrogen peroxide for prevention of future infection.

REFERENCES

1. Madhumathi D, Sakthi S. Bacterial contamination of toothbrushes in patients with gingivitis and healthy gingiva. International Journal of Pharmacy Research & Technology. 2019;9(2). doi: 10.31838/ijprt/09.02.05. DOI
2. El-Hamdaoui NE, Knezevic M, Knezevic M, Vicente-Barrero MM. Cross section study and analysis of toothbrushes contamination and disinfection. Study of 101 toothbrushes emloyed from the people of different ages. Head and neck. Russian Journal. 2020;8(2):45–51. doi: 10.25792/HN.2020.8.2.45–51. DOI
3. Gautam B, Pokhrel S, Aryal S, Basnet A. Efficacy of toothpaste in reducing micro-flora isolated from toothbrush. JSET. 2017;13(II):71-78. doi:10.3126/kuset.v13i2.21285. DOI
4. Frazelle MR, Munro CL. Toothbrush Contamination: A Review of the Literature. NRP. 2012. doi:10.1155/2012/420630. DOI
5. Glass RT. The infected toothbrush, the infected denture, and transmission of disease: a review. Compendium. 1992;13(7):592–598.
6. Mira A, Simon-Soro A, Curtis MA. Role of microbial communities in the pathogenesis of periodontal diseases and caries. JCPE. 2017;44(18):S23–S38. doi:10.1111/jcpe.12671. DOI
7. DeJuilio P, Powers J, Soltis LM, Brooks JA. Multisite evaluation of toothbrushes and microbial growth in the hospital setting. Clin Nurse Spec. 2023;37(2):83-89. doi: 10.1097/NUR.733. DOI
8. Karibasappa GN, Nagesh L, Sujatha BK. Assessment of microbial contamination of toothbrush head: an in vitro study. Indian J Dent Res. 2011;22(1):2-5. doi: 10.4103/0970-9290.79965.
9. Sharma S, Mohler J, Mahajan SD et al. Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment. Microorganisms. 2023;11:1614. doi:10.3390/microorganisms11061614. DOI
10. Orogu JO, Ehiwario NJ. Comparative study of bacteriological examination of daily use toothbrushes stored in the bathroom and room. EJSR. 2016;4(2):037-040. doi:10.18685/EJSR(4)2_EJSR-16-019. DOI
11. Pradeep S, Nandini G, Hiranmayi S et al. A Prospective Study on Assessment of Microbial Contamination of Toothbrushes and Methods of Their Decontamination. Cureus. 2022;14(10):e30155. doi 10.7759/cureus.30155.
12. Susheela P, Radha R. Studies on the microbiological contamination of toothbrushes and importance of decontamination using disinfectants. WJPMR. 2016;2:201–7.
13. Tomar P, Ganavadiya R, Hongal S et al. Evaluating sanitization of toothbrushes using ultra violet rays and 0.2% chorhexidine solution: A comparative clinical study. J Basic Clin Pharm. 2014;6(1):12-8. doi: 10.4103/0976-0105.145769. DOI
14. Peker I, Akca G, Sarikir C et al. Effectiveness of Alternative Methods for Toothbrush Disinfection: An In Vitro Study. TSWJ. 2014. doi: 10.1155/2014/726190. DOI
15. Collee JG, Fraser AG, Marmiom BP, Simmon A. Mackie and McCartenys Practical Medical Microbiology. 4th ed. Churchill Livingstone Inc., USA. 1996, pp.234-125.
16. MacFaddin JF. Biochemical tests for identification of medical bacteria (3rded.), Lippincott Williams and Wilkins, USA. 2000.
17. Joy T, Venugopal S, Sadanandan S, Mathew M. Evaluation of microbial contamination of toothbrushes and their decontamination using various disinfectants: An in vitro study. JIAPHD. 2022;20:200-5. doi: 10.4103/jiaphd.jiaphd_113_21. DOI
18. Ralephenya TRMD, Molepo J, Molaudzi M et al. Contamination of used toothbrushes and their decontamination with disinfecting agents. SADJ. 2020;75(9):478- 484. doi:10.17159/2519-0105/2020/v75no9a1. DOI
19. Otutu M, Igbineweka OM. Microbial contamination of tooth brushes and tooth brush keeping places among apparently healthy individuals in Egor LGA, Benin City. NJPASR. 2021;10(4):22-27.
20. Downes J, Samuel H, Melanie W, William W. Prevotella histicola sp. nov, isolated from the human oral cavity. IJSEM. 2008;58:1788-1791. doi: 10.1099/ijs.0.65656-0. DOI
21. Raiyani C, Arora R, Bhayya D et al. Assessment of microbial contamination on twice a day used toothbrush head after 1-month and 3 months: An in vitro study. JNSBM. 2015;6(3):44. doi: 10.4103/0976-9668.166072. DOI
22. Konidala U, Nuvvula S, Mohapatra A, Nirmala SV. Efficacy of various disinfectants on microbially contaminated toothbrushes due to brushing. Contemp Clin Dent. 2011;2:302-7. doi: 10.4103/0976-237X.91793. DOI
23. Naik R, Telagi N, Anil B, Spoorthi B. Contaminated tooth brushes-potential threat to oral and general health. J Family Med Prim Care. 2015;4(3):444-8. doi: 10.4103/2249-4863.161350. DOI
24. Nuvvula S, Mohapatra A, Nirmala SVSG, Konidala U. Efficacy of various disinfectants on microbially contaminated toothbrushes due to brushing. Contemp Clin Dent. 2011;2(4):302-7. doi: 10.4103/0976-237X.91793. DOI
25. Strassle PD, Sickbert-Bennett EE, Klompas M et al. Incidence and risk factors of non-device-associated pneumonia in an acute-care hospital. Infect Control Hosp Epidemiol. 2020;41(1):73-79. doi: 10.1017/ice.2019.300. DOI
26. Almutairi T, Aldossary A, Alshammari A, Alwakeel S. Investigations into the Microbial Contamination of Toothbrushes Isolated from Riyadh, Saudi Arabia. Advances in Environmental Biology. 2014;8(7):2231–5.
27. Quinn B, Baker DL, Cohen S et al. Basic nursing care to prevent nonventilator hospital-acquired pneumonia. J Nurs Scholarsh. 2014;46(1):11-9. doi: 10.1111/jnu.12050. DOI

28. Munro S, Baker D. Reducing missed oral care opportunities to prevent non-ventilator associated hospital acquired pneumonia at the Department of Veterans Affairs. Appl Nurs Res. 2018;44:48-53. doi: 10.1016/j.apnr.2018.09.004. DOI
29. Munro S, Haile-Mariam A, Greenwell C et al. Implementation and dissemination of a Department of Veterans Affairs oral care initiative to prevent hospital-acquired pneumonia among nonventilated patients. Nurs Adm Q. 2018;42(4):363–372. doi: 10.1097/NAQ.0000000000000308. DOI

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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